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Variation in courtship signals and hybridization between geographically definable populations of the rice Brown planthopper, *Nilaparvata lugens* (Stål)

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Populations of *Nilaparvata lugens* from 18 geographically defined and widely distributed regions in Asia and Australasia were maintained in the laboratory on growing rice plants. Crosses between some of these showed varying degrees of success in hybridization. Those between insects from Australia and the Solomon Islands had the lowest success rates, but in successful individual crosses there was little evidence of hybrid inviability. Behavioural barriers in the form of substrate transmitted courtship signals appeared to be primarily responsible for low success in hybridization. Pulse repetition frequencies of male calls were distinctive for different populations: those from Australia and the Solomon Islands showed the greatest difference. Divergence in mate recognition signals (pre-mating ethological isolating mechanisms) has apparently evolved in advance of general genetic incompatibilities (post-mating isolating mechanisms) in this species.

KEY WORDS: – Brown planthopper – *Nilaparvata lugens* – rice – hybridization – courtship – acoustic signals – geographical variation – isolating mechanisms – mate recognition.

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INTRODUCTION

Nilaparvata lugens (Stål) is widely distributed in south and east Asia, northern Australia and western Oceania (Fig. 1). For host plants it is effectively restricted to cultivated and wild rices, *Oryza* species (Mochida & Okada, 1979). Eggs are laid beneath the leaf sheaths of a rice plant. Nymphs and adults feed on the

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Figure 1. Sketch map of Asia to show sites of origin of culture populations of *N. lugens*. Numbers represent localities as in Table 1.

phloem sap which they suck usually from the basal regions of the plant. Adults may be either fully winged macropters or short winged brachypters. *Nilaparvata lugens* is currently one of the most important and damaging pests of rice in Asia (International Rice Research Institute, 1979).

A major strategy for the control of *N. lugens*, developed to great effect at the International Rice Research Institute (I.R.R.I.), has been the use of varieties of rice bred for their resistance to attack by the insect (Khush, 1979). A number of genes have been identified in cultivars and wild species of rice which show resistance to at least some populations of *N. lugens*. Most such resistance factors derive from cultivars from India and Sri Lanka. In turn, some populations of the insect have developed the ability to damage such resistant varieties—that is, they have developed virulence to them. Populations with distinct patterns of virulence have often been termed 'biotypes' (International Rice Research Institute, 1979). However, Claridge & Den Hollander (1980) and Den Hollander & Pathak (1981) have shown that such virulence characters are highly labile and overlap between the so-called biotypes. Indeed, Claridge & Den Hollander (1982) have shown that biotypes 1, 2 and 3 from the Philippines,

virulent respectively to varieties with no gene for resistance (e.g. TN1), with gene *Bph* 1 (e.g. Mudgo) and with *bph* 2 (e.g. ASD7), after as few as 10 generations of selection on a variety with a different resistance gene, may be effectively changed to another biotype. Pathak & Heinrichs (1982) have independently obtained similar results by selection experiments on biotypes 2 and 3. It is clear then that the three biotypes of *N. lugens* at I.R.R.I. simply represent sympatric populations selected for different virulence characteristics. Similar patterns of virulence may also be achieved independently in allopatric populations (Claridge & Den Hollander, 1982; Claridge, Den Hollander & Furet, 1982). Thus variation in virulence characters is not generally suitable as a basis for studying geographical variation and genetic diversity in this species.

It is known that populations of *N. lugens* in some of the northern temperate areas of its range, such as Japan and Korea, are unable to survive the winters in those regions. Such populations depend on annual renewal by long-distance migrations of insects from more southerly tropical or subtropical areas during the spring and early summer (Kisimoto, 1979). However, the role of long-distance migration within the tropical parts of the species range is uncertain and little studied. If regular migration and intermixing of populations takes place on a large geographical scale in the tropics, then it might be expected that local populations of the insect would show little genetic differentiation. However, if such populations were often truly allopatric then local adaptation might be expected to have caused evolutionary divergence between them. Thus the study of geographical variation in *N. lugens* may give us a clue to the importance of long range migration in the invasion of tropical rice crops.

Nilaparvata lugens is a distinct morphospecies, easily separated from the other four species of *Nilaparvata* presently known from the Oriental and Australasian regions on a basis of morphological characters, mainly of the male genitalia (Mochida & Okada, 1979). Nevertheless, the species shows considerable morphological variation within populations, some of which is undoubtedly environmentally induced by such factors as crowding and food quality. Analyses of morphological and morphometric differences between populations are difficult to interpret without information on the heritability of the characters studied.

A more helpful approach may be to study hybridization between different populations in order to get some idea of levels of genetic differentiation. Here we present data on hybridization experiments between populations from different regions within the overall area of distribution of *N. lugens*, together with analyses of variation in acoustic signals in the same populations.

MATERIALS AND METHODS

We have imported populations of *N. lugens* to Cardiff from widely dispersed regions of Asia and Australasia (Fig. 1). Details of populations discussed in this paper are given in Table 1. Insects were cultured on growing rice plants, usually of the same variety from which they were collected, in wooden frame gauze covered cages ($1 \times 0.25 \times 0.25$ m), in a glass house maintained at a temperature of $25 \pm 2°C$ and a daylength of at least 12 h. For hybridization and other experiments, insects were isolated in cylindrical mylar cages which were designed each to fit over a single potted rice plant.

Table 1. Detailed localities, latitude and longitude, and year of import to Cardiff of culture populations of *N. lugens*

	Locality			Latitude	Longitude	Year of import
1.	India	West Bengal	Chinsurah	22.53N	88.25E	1981
2.	Bangladesh		Dacca	23.42N	90.22E	1978
3.	Sri Lanka		Kandy	7.17N	80.40E	1978
4.	Thailand		Bangkok	13.44N	100.30E	1982
5.	China	Sichuan	Chongqing	29.30N	106.35E	1981
6.	Japan	Honshu	Omagari	39.29N	140.29E	1977
7.	Philippines	Luzon	Los Baños (Biotype 1)	14.10N	121.26E	1979
8.	Philippines	Luzon	Los Baños (Biotype 2)	14.10N	121.26E	1979
9.	Philippines	Luzon	Los Baños (Biotype 3)	14.10N	121.26E	1979
10.	Philippines	Luzon	Liliw	14.7N	121.00E	1979
11.	Philippines	Mindanao	Davao	7.05N	125.38E	1978
12.	Malaysia	Selangor	Serdang	5.16N	100.34E	1979
13.	Indonesia	Sumatra	Metro	5.08N	105.15E	1982
14.	Indonesia	Java	Jogyakarta	7.48N	110.24E	1982
15.	Papua New Guinea		Lae	6.45S	147.00E	1982
16.	Solomon Islands	Guadalcanal	Honiara	9.28S	159.57E	1982
17.	Australia	Western Australia	Kununurra	15.42S	128.50E	1982
18.	Australia	Northern Territory	Darwin	12.23S	130.44E	1982
19.	Australia	Queensland	Mareeba	17.00S	145.28E	1982
20.	Australia	Queensland	Ayr (1)	19.32S	147.25E	1976
21.	Australia	Queensland	Ayr (2)	19.32S	147.25E	1979
22.	Fiji	Viti Levu	Suva	18.08S	178.25E	1982

Hybridization experiments

Previously we used mate choice experiments to test success in hybridization between populations of *N. lugens* (Claridge & Den Hollander, 1980). However, in such tests it is often difficult to be sure when successful copulation as opposed to attempted copulation has taken place. Therefore here we have used crosses between individual virgin males and females to investigate success in insemination between different populations. Late fifth instar female nymphs were isolated and allowed to moult into adults. After 4–5 days in isolation, each female was placed in a 8×1 inch glass tube containing a rice seedling with an individual male. After 24 h the female was removed from the tube and dissected to examine the spermatheca for the presence of living sperm. This proved quite a simple operation and gave a measure of successful mating.

Similar crosses were set up and kept for examination of offspring and to determine whether or not eggs had been laid and rates of egg hatch.

Courtship signals

The substrate transmitted acoustic signals produced by males and females of *N. lugens* were initially recorded by use of a simple crystal gramophone cartridge (Acos. GP 91-3SC). More recently we have used an accelerometer (Brujel & Kjaer 8302) and charge amplifier, type 2635, for the same purpose (Fig. 2), because of its flatter frequency response and relative insensitivity to external sounds. In both cases the signals were amplified and recorded on magnetic tape using a Nagra 4.2 LSP tape recorder at a tape speed of 38.1 cm^{-1} (15 inch s^{-1}).

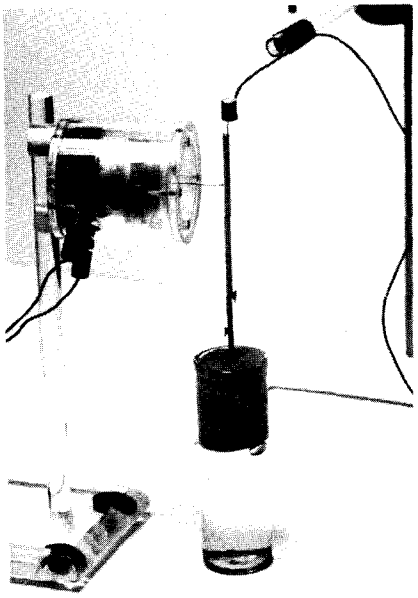


Figure 2. Apparatus used to detect and play-back acoustic signals of *N. lugens*. A small rice plant is shown with two insects. The accelerometer is attached to the top of the plant. A modified loudspeaker used for playback makes light contact with the stem. See text for details.

Oscillograms for the analysis of such recordings were made using a Siemens Mingograph 34. Recorded calls were played back into the plant by using a small loudspeaker with the paper cone removed and a needle firmly attached to the moving coil (Fig. 2). All signals were recorded at a temperature of $25 \pm 2°C$.

RESULTS AND CONCLUSIONS

Hybridization

Pairings were established between individual males and females from different populations, and successful inseminations are summarized in Table 2. First, crosses between the three sympatric Philippine 'biotype' populations all showed very high levels of successful matings. Other crosses were established between a Philippine population, for which 'biotype 3' was used, and various other geographically definable populations. Some of these crosses also showed high levels of successful mating, but others, especially those involving Australian populations, gave low levels of success—below 30% for both combinations of sexes. Also, some crosses between Philippine and Solomon Islands insects gave poor levels of success. Some other crosses were made, which did not include one parent from the Philippines. Again, those involving Australian insects showed low levels of success in mating. These preliminary crosses clearly suggested that there were some barriers to mating between some of the populations of *N. lugens* tested. The greatest problems arose in crosses involving the populations from Australia and the Solomon Islands.

Because they showed evidence of greatest incompatibilities in hybridization experiments, populations from Australia, the Solomon Islands and the

Table 2. Numbers of individual crosses made between different culture populations of *N. lugens* and percentages of successful crosses as measured by observed inseminations

Cross	No. of crosses	Percentage success
Philippine biotypes		
Biotype 3 female × Biotype 3 male	25	100
Biotype 3 female × Biotype 1 male	25	96
Biotype 3 male × Biotype 1 female	25	92
Biotype 3 female × Biotype 2 male	25	100
Biotype 3 male × Biotype 2 females	25	100
Crosses involving at least one Philippine parent		
Philippine female × Philippine (Liliw) male	25	88
Philippine female × Solomon Is. male	25	92
Philippine male × Solomon Is. female	25	52
Philippine female × Japan male	25	75
Philippine male × Japan female	17	88
Philippine female × Australian male	38	16
Philippine male × Australian female	38	26
Philippine female × Bangladesh male	27	96
Philippine male × Bangladesh female	25	88
Philippine (Liliw) male × Australian female	37	3
Philippine (Liliw) female × Australian male	75	37
Philippine (Liliw) male × Solomon Is. female	12	100
Philippine (Liliw) female × Solomon Is. male	21	38
Other crosses		
Australian female × Australian male	34	94
Australian female × Solomon Is. male	118	4
Australian male × Solomon female	137	7
Australian male × Japan female	21	0
Solomon Is. female × Sri Lanka male	28	61
Solomon Is. male × Sri Lanka female	24	67

Philippines were used in a further series of crosses to estimate success in hybrid development in those crosses in which insemination was successful. For these experiments the first Australian population from Ayr (Table 1, no. 20), the Liliw population from the Philippines (Table 1, no. 10) and the Solomon Islands population (Table 1, no. 16) were used. Crosses which resulted in no hatched eggs were discarded since it was assumed that the ovipositing females were unmated. This was confirmed for some crosses by examining the spermatheca for the presence of sperm after the experiments. No such females were found to be inseminated. Crosses which resulted in 10 eggs or less were also discarded. The percentages of eggs hatched were calculated for each successful cross (Table 3). There were no significant differences between these and control crosses from the same populations. Thus, it may be concluded that, though strong barriers to hybridization exist between the three populations studied, when crosses do take place, numbers of progeny are not significantly different from intra-population crosses.

In a series of crosses between a Philippine population and an Australian one produced for studying courtship signals (see below), the F_1 generations showed normal sex ratios, and no problems were encountered in obtaining F_2

Table 3. Percentages of eggs hatching in crosses involving populations of *N. lugens* from the Philippines, Australia and Solomon Islands. Egg hatch was also measured for two crosses between F_1 parents

Cross	No. of crosses	No. of eggs	Percentage hatch	Percentage hatch F_1
Philippine × Philippine	16	1410	61	—
Australia × Australia	15	1210	64	—
Solomon Is. × Solomon Is.	9	743	83	—
Philippine males × Australian female	29	4835	74	84
Philippine female × Australian male	24	2051	75	—
Philippine male × Solomon Is. female	19	1279	61	—
Philippine female × Solomon Is. male	21	1682	67	—
Australia male × Solomon Is. female	20	2099	67	81
Australia female × Solomon Is. male	29	3298	81	—

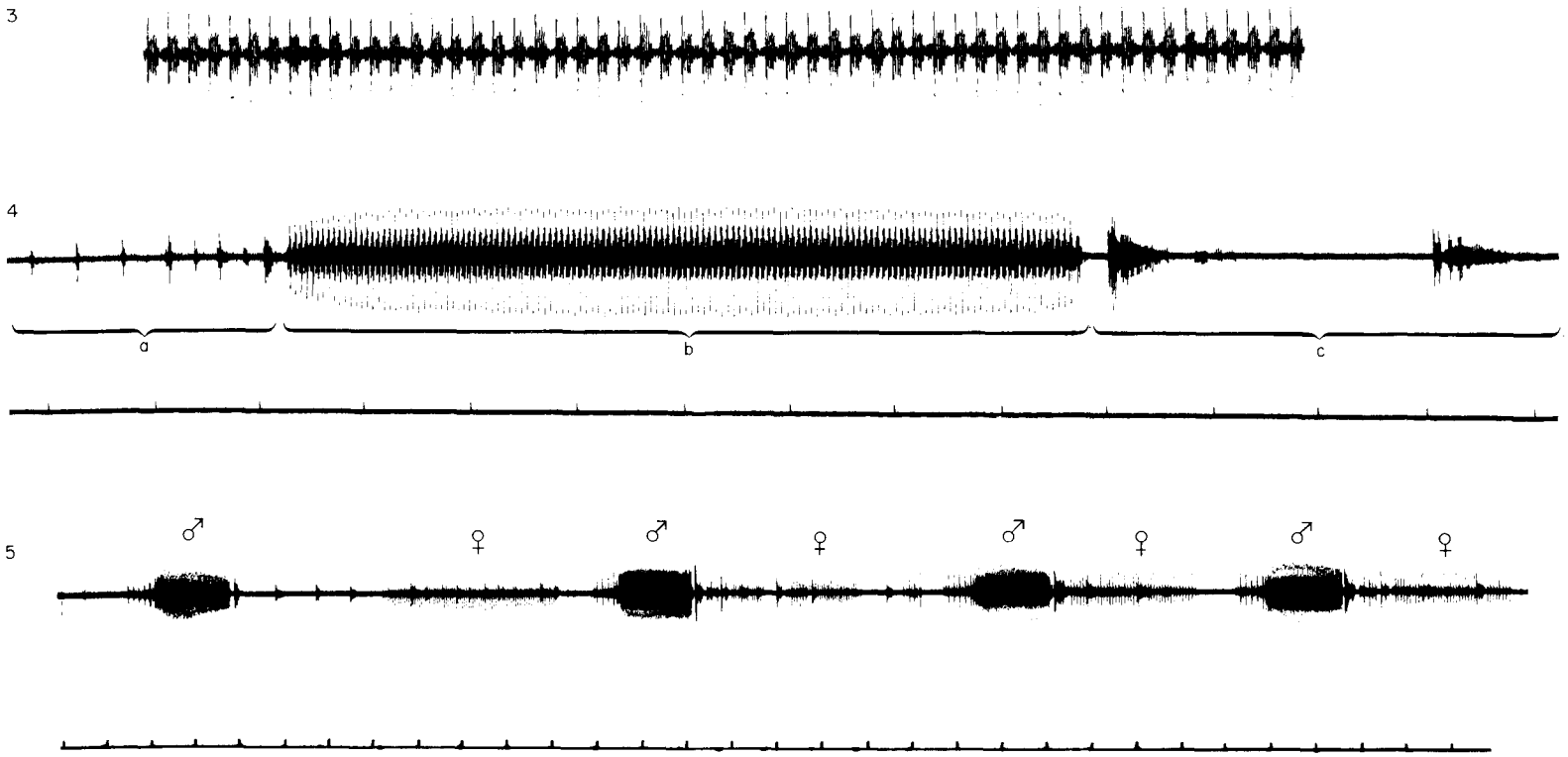
generations. It thus appears that genetic incompatibility between the two populations is not great.

Courtship signals

Adults of *N. lugens* communicate primarily by means of acoustic signals transmitted through the substrate (Ichikawa & Ishii, 1974). In this, they resemble most of the smaller Auchenorrhyncha (Ossiannilson, 1949; Claridge, 1983). Courtship behaviour may be initiated by either sex. The female call consists of regularly repeated simple pulses (Fig. 3) produced by a visible vibration of the abdomen (Ichikawa & Ishii, 1974). The male call, which may be produced in response to that of the female, has a more complicated structure (Fig. 4). It consists of repeated sections which themselves consist typically of three phases, (a) a series of from three to 10 complex pulses, (b) a series of regularly and rapidly repeated pulses, and (c) further complex groups of pulses. Phases a and c are very variable and phase c may often be lacking. Phase b is the most obvious and consistent element of the male song. Sexually receptive males of *N. lugens* respond to the female call by rapidly moving about the plant, and at the same time themselves calling. This usually results in an alternation of male and female calls (Fig. 5). Eventually, the male makes contact with the female and mating may take place. It is clear that acoustic signals are of major importance in bringing the sexes together, and presumably therefore in species recognition. This was demonstrated simply by playing back recorded songs through a small modified loudspeaker on to a plant on which live insects had been placed. In this way, it was possible to release calling and mate-searching behaviour in receptive males without the presence of a live female.

We recorded individual male acoustic signals for samples from 22 populations of *N. lugens* and measured pulse repetition frequencies (P.R.F.) for phase b of each call. Means for each of the 18 geographically definable populations were calculated (Fig. 6). Maximum non-significant ranges of population means at the 5% level of significance were computed for the full 22 populations using the Duncan procedure for making comparisons amongst means (Table 4).

In all comparisons the populations from Australia were significantly different



Figures 3–5. Oscillograms of parts of calls of adult *N. lugens*. Fig. 3. Female. Fig. 4. Male. Fig. 5 Male and female interaction. Time marks at 0.25 s intervals, Figs 3 and 4 to same scale.

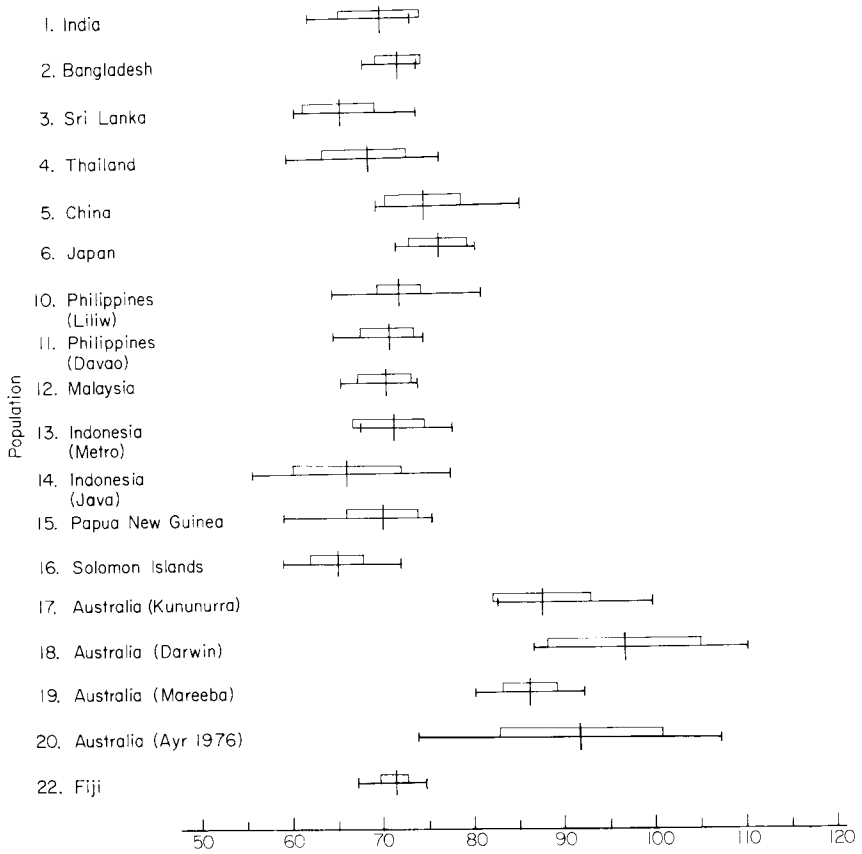


Figure 6. Analyses of pulse repetition frequencies (P.R.F.) of main phases of male calls of *N. lugens* populations. Vertical line represents population mean, closed box one standard deviation on either side of the mean, and horizontal line range of individual means.

from all others, thus confirming our previous preliminary comparison between one Australian population and one from the Philippines (Claridge *et al.*, 1982). In addition the population from Darwin was itself significantly different from all others, including the others from Australia.

The Duncan procedure grouped the remaining populations from localities other than Australia into five partly overlapping subsets (Table 4). Thus the Solomon Islands population was grouped only with those from Java and Sri Lanka. The two latter were in turn linked to five other populations. All remaining populations belonged to widely overlapping subsets.

Although only the populations from Australia showed complete separation from all others, this analysis demonstrated the existence of lesser degrees of differentiation between some of the other populations. The biological significance of these differences are not certain and no obvious geographical pattern of variation is apparent. Great caution should be used in interpreting the smaller differences amongst these data.

Because of their distinctness at the two extremes of our range of P.R.F. measurements of male calls, most of our hybridization experiments have so far

Table 4. Numbers of individuals (N) recorded and ranked sample means ($\bar{x}$) for pulse repetition frequencies of male calls from 22 populations of *N. lugens* (See Table 1). Vertical lines link subsets of samples with maximum non-significant ranges of means ($P = 0.05$) computed by the Duncan procedure for multiple comparison of means

N	Mean P.R.F. ($\bar{x}$)	Population	Maximum non-significant ranges
24	64.94	16. Solomon Islands	]
16	66.00	3. Sri Lanka	]
18	66.32	14. Java	]
22	68.88	22. Fiji	]
26	69.03	4. Thailand	]
24	70.22	15. Papua New Guinea	]
12	70.28	1. India	]
9	70.56	12. Malaysia	]
10	71.09	11. Phil. Davao	]
25	72.08	13. Sumatra	]
16	72.18	9. Phil. Biotype 3	]
21	72.48	10. Phil. Liliw	]
11	72.74	2. Bangladesh	]
16	74.34	7. Phil. Biotype 1	]
12	75.24	5. China	]
16	76.32	8. Phil. Biotype 2	]
13	76.69	6. Japan	]
		Australia	
23	86.23	19. Mareeba	]
14	87.74	21. Ayr 2	]
6	87.76	17. Kununurra	]
38	91.79	20. Ayr 1	]
11	96.96	18. Darwin	]

used populations from Australia and the Solomon Islands, together also with one from the Philippines, as representing a population with an intermediate P.R.F.

It might be argued that the differences between our culture populations do not reflect real field differences, but may be either artefacts of original sampling errors, or effects of selection or random processes operating within our relatively small sample populations. However, wherever possible we have imported at least two separate culture populations from the same or nearby areas and recorded them for comparison. For example, we made two separate importations from Ayr in Queensland, Australia (Table 1, nos. 20, 21). These did not differ significantly in male P.R.F. (Table 4). Similarly, four populations from Luzon, northern Philippines, including the three I.R.R.I. biotype cultures (Table 1, nos. 7–9) and a field population from Liliw (Table 1, no. 10) were also recorded. The four populations were closely grouped in two overlapping subsets for measurements of P.R.F. (Table 4). Thus there is good evidence to suggest that the acoustic differences between culture populations do represent some real differences between field populations.

It may be concluded from these results, therefore, that the male calls of *N. lugens* may show significant geographical variation. However, it could be

argued that these differences might be environmentally induced and therefore do not reflect genetic differentiation between the populations. In order to test this further, we obtained a hybrid population by crossing an Australian population with one from the Philippines. Males and females of a F_1 hybrid generation were obtained. Measurements of pulse repetition frequencies for male hybrids gave results intermediate between and significantly different from both parental populations (Fig. 7). This would be expected if such frequencies were largely under genetic control. F_2 hybrids of both sexes were also successfully obtained. The male calls of these were similar to and did not differ significantly from those of the F_1 (Fig. 7). They showed no evidence of segregation, thus suggesting a polygenic system of inheritance for the calls.

The studies of male courtship calls support the suggestion from hybridization studies that at least some geographically defined populations of *N. lugens* are characterized by different mean pulse repetition frequencies. Smaller differences between other populations may also be biologically significant, but here we emphasize only the most distinct ones. We have made similar studies on pulse repetition frequencies of the simpler female calls of three populations of *N. lugens* from Australia, the Philippines and the Solomon Islands. Comparisons between the means showed some small but significant differences (Fig. 8) and further analyses of other populations are required.

Thus, those populations which we have found most difficult to hybridize in the laboratory, for example those from Australia, Solomon Islands, and the Philippines, also show the greatest differences in acoustic signals. When hybrids are obtained between any of these groups of populations they show little indication of hybrid inviability and we suggest that the differences in the calls themselves probably account for the low success in hybridization between the populations. It should be emphasized that we have not yet made crosses

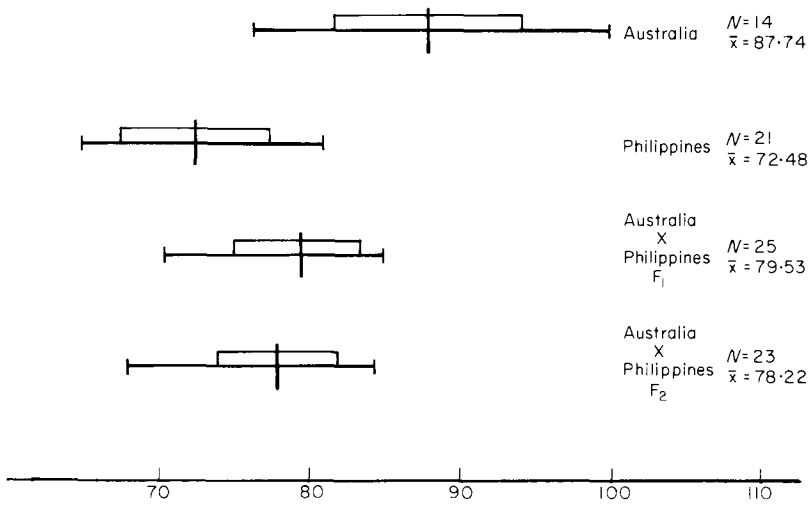


Figure 7. Analyses of pulse repetition frequencies of male calls for parental populations of *N. lugens* from Philippines and Australia and F_1 and F_2 hybrid populations from crosses between them, with numbers of individuals (N) recorded and sample means ($\bar{x}$). Conventions as in Fig. 6.

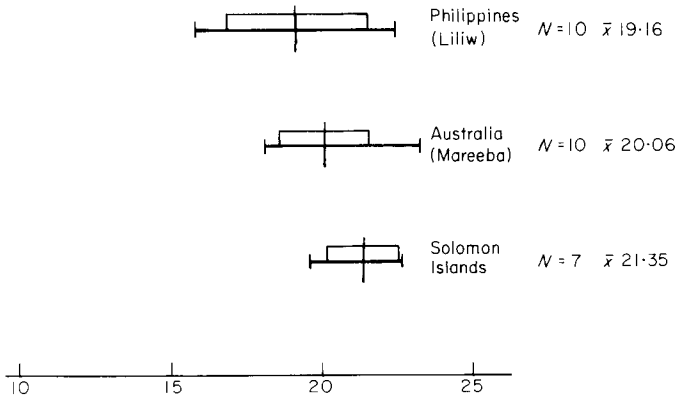


Figure 8. Analyses of pulse repetition frequencies of female calls for populations of *N. lugens* from Australia, Solomon Islands and Philippines, with numbers of individuals recorded (N) and sample means ($\bar{x}$). Conventions as in Fig. 6.

between all populations maintained in culture so that other significant differences may also occur. This indeed is clearly suggested from the analysis of other male calls (Table 4, Fig. 6).

DISCUSSION

The problems of establishing the evolutionary status of allopatric populations are familiar and have been widely discussed in the past (e.g. Cain, 1954; Mayr, 1942, 1963). In principle it is not possible to determine whether two allopatric populations of related bisexual organisms would inter-breed and coalesce if their breeding ranges were to expand and overlap in nature. Nevertheless, some useful information may be obtained by laboratory hybridization experiments. If two allopatric populations can be shown either to be incapable of producing hybrids or to be capable of producing only sterile hybrids in the laboratory, then it is reasonable to assume that the same results would obtain in the field and that the two populations represent genetically distinct biological species. However, if two populations hybridize relatively freely in the laboratory, then it is more difficult to interpret the results.

Two populations of *N. lugens* discussed in this paper, from the Philippines and from Australia, are naturally allopatric, and when crossed in the laboratory showed low levels of successful hybridization. However, in those individual crosses which were successful, there were no obvious indications of hybrid inviability. It thus seems that there is some pre-mating barrier which in most experiments prevented mating. These populations clearly represent an early stage in the process of speciation.

The role of substrate transmitted signals in the courtship behaviour of *N. lugens* and two other rice-feeding planthoppers, *Sogatella furcifera* (Horvath) and *Laodelphax striatellus* (Fallén) was first elegantly demonstrated by Ichikawa, Sakuma & Ishii (1975). They showed that in Japan the calls served as isolating mechanisms between these rather distantly related sympatric species.

Within the species *N. lugens* it is clear that the alternating courtship calls of males and females act as specific mate recognition signals (in the sense of

Paterson, 1982). It would be expected that male and female acoustic receptors should be tuned to respond to the appropriate calls of the opposite sex. It is clear that this is so, for example, for the allopatric populations from the Philippines and Australia. The calls of these populations differ most obviously in the pulse repetition frequencies of the major section of the male signals (Fig. 6). It seems very likely that in these populations such calls have diverged in isolation to such a point that difficulty is experienced in achieving successful hybridization when they are brought together in the laboratory. It seems then that these isolated populations have diverged more rapidly in male courtship signals than in general genetic incompatibility. In the terminology of Mayr (1963) pre-mating ethological isolating mechanisms have begun to evolve in advance of post-mating mechanisms.

The primary divergence of populations in isolation is an essential first stage in all allopatric models of speciation. It is generally assumed that isolated populations diverge primarily in genetic factors which incidentally lead to incompatibilities of any hybrids produced between them. Such incompatibilities would be the developing 'post mating isolating mechanisms' of Mayr and other authors. Generally it might be expected that 'pre-mating mechanisms' or 'specific mate recognition signals' should be buffered against divergence in isolation as a result of the operation of intense stabilizing selection. Indeed, it is widely held that behavioural isolating mechanisms or specific mate recognition signals normally show little or no geographical variation (e.g. Moore, 1957; Paterson, 1981, 1982).

Until recently few studies have specifically set out to look for geographical variation in courtship signals. Because of the ease with which acoustic signals may be analysed in detail it might be expected that, if such variation occurs at all commonly, then animals which use such signals might be good ones to investigate. Amongst vertebrates the most widely studied for their acoustic signals are birds (e.g. Marler, 1960; Thielke, 1969) and amphibians (e.g. Bogert, 1960). Here many examples of local variation in calls have been described but these may often be mainly learned variations and not necessarily genetically determined. Amongst insects, where offspring rarely have the possibility of learning details of calls from their parents, such call differences are more likely to be primarily genetically determined. The classic studies of Perdeck (1958) on grasshoppers and Hoy (1974) on crickets clearly demonstrated this.

Few insects have been studied in detail over a sufficiently wide area of distribution to demonstrate possible variation in courtship calls. Walker (1974) assessed the evidence for such variation in the best studied acoustic insects of N America: the crickets and bush crickets. Though the main purpose of his paper was to review the evidence for character displacement in such calls, incidentally he documented a number of examples of significant variation in song characteristics in allopatric populations of some of these insects. More recently Ikeda, Takabatake and Sawada (1980) have described significant quantitative differences in interpulse intervals of male courtship signals from interfertile populations of *Drosophila mercatorum* from New York, El Salvador and Hawaii. These results closely resemble those reported here on variation in pulse repetition frequencies of the male courtship calls of *N. lugens*.

As in *Drosophila mercatorum*, so in *N. lugens*, the most obvious differences between allopatric populations concern quantitative variations in male

courtship signals. The most extreme differences were found between Australian populations and those from the remaining regions, but significant differences were found between some others (Table 4). There is no indication of great genetic differentiation between any of the populations studied, though some biochemical differences between some populations have been demonstrated by gel electrophoresis techniques (Claridge, Den Hollander & Morgan, 1983).

It is remarkable that divergence in specific mate recognition signals should occur in allopatric populations in advance of other major evolutionary changes. Indeed variation in pulse repetition frequencies of the male calls of *N. lugens* provides the most sensitive measure of geographical divergence between populations which we have yet found. It is not possible at present to suggest the nature of the evolutionary pressures which may be responsible for such variation. Also, more work on other species is required to establish how unusual are our results with *N. lugens* and those of Ikeda *et al.* (1980) on *Drosophila mercatorum*. Booij (1982) has found some evidence of similar quantitative variation in calls of geographically isolated populations of the planthopper genus *Muellerianella* in Europe. It is possible that the widely held assumption about the nature of initial allopatric divergence of populations during speciation may require some modification.

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